453. *Chemistry of the Higher Fungi. Part XI.* Polyacetylenic Metabolites of* Drosophila subatrata.

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Five of the many polyacetylenic metabolites of the Basidiomycete *Drosophila* (*Psathyrella*) *subatrata* have been isolated in a crystalline state and investigated in detail. Drosophilin C is shown to be the *cis*-ethylenic triacetylenic C_{11} acid (I), containing the novel $HC{\equiv}C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}$ system, converted by sodium hydrogen carbonate at 20° into drosophilin D (III) with a terminal allenic group. Drosophilin D also occurs in the fungal cultures and both compounds isomerise in 10% aqueous sodium carbonate to the conjugated ene-triyne acid (IV), still containing the *cis*-ethylenic linkage. Drosophilin E is the C_9 acid (VII). The more polar fractions contain the dicarboxylic acids (VIII) and (IX); the latter is exceptional in containing an odd number of carbon atoms and no free ethynyl (or equivalent) group.

The Basidiomycete fungus *Drosophila* (*Psathyrella*) *subatrata* was originally investigated by Kavanagh, Hervey, and Robbins [1] in 1952 and by Anchel.[2] Several metabolic products were recognised and characterised by their absorption spectra, including two polyacetylenic acids termed drosophilin C and D. The ultraviolet absorption data quoted are compatible with the presence of an ene-diyne chromophore in the former and probably an ene-diyn-ene system in drosophilin D. Isomerisation of both compounds with alkali resulted in the production of materials with identical absorption, *i.e.*, that of an ene-triyne system. The spectral changes when drosophilin C was treated with alkali are strongly reminiscent of the isomerisation of nemotin.[3]

We have grown *Drosophila subatrata* in surface culture on a malt medium; fractionation of the ethereal extract of the culture fluid showed that a very complex mixture of polyacetylenes was present. Extensive counter-current distributions between phosphate buffers and ether–benzene mixtures eventually resolved this mixture into five main fractions. The first four, in order of increasing polarity, consisted mainly of drosophilin F, D, C, and E while the fifth, a much more polar fraction, was still a complex mixture of

* Part X, *J.*, 1960, 691.

[1] Kavanagh, Hervey, and Robbins, *Proc. Nat. Acad. Sci. U.S.A.*, 1952, **38**, 555.
[2] Anchel, *Arch. Biochem.*, 1953, **43**, 127.
[3] Bu'Lock, Jones, Leeming, and Thompson, *J.*, 1956, 3767.

polyacetylenes. The simpler procedures of Kavanagh *et al.*[1] had resulted in the isolation of only two polyacetylenes, drosophilin C and D.

(I) $HC{\equiv}C{\cdot}CH_2{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{c}CH{\cdot}CH_2{\cdot}CO_2H$

(II) $HC{\equiv}C{\cdot}CH_2{\cdot}CH{=}^{c}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CO_2H$

(III) $H_2C{=}C{=}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{c}CH{\cdot}CH_2{\cdot}CO_2H$

(IV) $CH_3{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{c}CH{\cdot}CH_2{\cdot}CO_2H$

(V) $H_2C{=}C{=}CH{\cdot}CH{=}^{c}CH{\cdot}C{\equiv}C{\cdot}C{\equiv}C{\cdot}CH_2{\cdot}CO_2H$

(VI) $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}C{=}CH{\cdot}CH{=}CH{\cdot}CH_2{\cdot}CO_2H$

(VII) $HC{\equiv}C{\cdot}C{\equiv}C{\cdot}CH{=}^{c}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$

Drosophilin C formed stable needles, gave analyses for $C_{11}H_8O_2$, and yielded undecanoic acid on hydrogenation. Its ultraviolet absorption suggested a disubstituted ene-diyne chromophore while the infrared spectrum revealed the presence of free acetylenic hydrogen, *cis*-ethylenic hydrogen, and an unconjugated carboxyl group, the last fact being confirmed by the formation of an unconjugated methyl ester (infrared spectrum) with methanol and sulphuric acid. These facts are compatible only with structure (I) or (II) for drosophilin C and a study of its behaviour on alkali-isomerisation permitted a choice to be made between them.

Drosophilin C was found to undergo a two-stage isomerisation in alkali. In sodium hydrogen carbonate solution, the ultraviolet absorption spectrum rapidly changes and the reaction is complete in 2 hours. The product forms colourless, low-melting needles and the new chromophore is typical of an ene-diyne-ene (longest-wavelength maximum at 3085 Å). The infrared absorption spectrum indicated an unconjugated acid (confirmed by the formation of a methyl ester with carbonyl absorption at 1745 $cm.^{-1}$). There was no free acetylenic hydrogen band, but bands at 1960, 1930, and 838 $cm.^{-1}$ indicated a terminal allenic structure.[4] *cis*-Ethylenic hydrogen absorption was also present. Hydrogenation followed by reduction with lithium aluminium hydride gave undecanol. These facts establish the structure (III) unequivocally for the isomerisation product.

A second stage of isomerisation occurs in 10% sodium carbonate solution or, more rapidly, in sodium hydroxide solution. The product is a colourless crystalline compound which readily forms a methyl ester. The ultraviolet absorption spectrum is typical of the ene-triyne chromophore and bands were present in the infrared spectrum showing *cis*-ethylenic hydrogen and an unconjugated carboxyl group. No bands attributable to free acetylenic hydrogen or an allene were present. Undecanoic acid was formed on hydrogenation and structure (IV) follows for this product which is the " drosophilin C alk " of Anchel.[2]

These isomerisations make it clear that drosophilin C is correctly represented by (I). A compound of structure (II) would probably isomerise in alkali but with difficulty, and the product would be the diene-diyne (V) which would show ultraviolet absorption [5] quite different from that of either of the isomerisation products actually obtained; the acid (V) would not be expected to isomerise further unless very strongly basic conditions were used.[6]

Several attempts were made to convert acids (I), (III), and (IV) or their esters into the known *trans*-isomer [7] of (IV) by more vigorous alkali-treatment. All such experiments resulted largely in destruction of the polyacetylenes and any surviving ene-triyne material still showed *cis*-double-bond absorption in the infrared spectrum.

Mention must be made of the structure (VI) which has recently been proposed as a possibility for drosophilin C.[8] Such a structure is now precluded on three grounds. Drosophilin C contains no allenic group. A compound of structure (VI) would show strong

[4] Wotiz and Mancuso, *J. Org. Chem.*, 1957, **22**, 207.
[5] Bohlmann and Herbst, *Chem. Ber.*, 1958, **91**, 1631.
[6] Bertrand, *Compt. rend.*, 1958, **247**, 824.
[7] Bohlmann and Viehe, *Chem. Ber.*, 1954, **87**, 712.
[8] Anchel, *Science*, 1957, **126**, 1229.

diene absorption in the 2300 Å region superimposed upon the ene-diyne spectrum and it would isomerise directly to (IV) without affording any isolable intermediate (ref. 9).

Drosophilin D proved exceedingly difficult to purify, three successive counter-current distributions giving only an oil. Crystallisation was eventually accomplished (see p. 2261) and drosophilin D proved to be identical with the product (III) from the first stage of isomerisation of drosophilin C. Isomerisation in sodium carbonate solution gave the ene-triyne acid (IV), the same as the product obtained from drosophilin C.

Drosophilin E was discovered as an impurity contaminating the later drosophilin C fractions in the counter-current purification of *Drosophila* extracts. The two compounds are of closely similar polarity and no distribution system could be found which effected complete separation. Drosophilin E is unaffected by treatment with 0·1N-sodium hydroxide and hence fractions containing C mixed with E showed incomplete isomerisation when treated with base. This principle was used to follow the distribution of drosophilin C (see p. 2261). Further, the products of isomerisation of drosophilin C are appreciably less polar than drosophilin E, so that when a mixture of C and E was treated with sodium hydrogen carbonate solution the resulting drosophilin D and E were readily separated by counter-current distribution.

Drosophilin E was rather less stable than C and D. It formed colourless prisms giving fairly good analyses for $C_9H_8O_2$. The ultraviolet absorption spectrum indicated a mono-substituted ene-diyne chromophore while the infrared spectrum revealed the presence of free acetylenic hydrogen, *cis*-ethylenic hydrogen, and an unconjugated carboxyl group. Since hydrogenation gave nonanol these data establish its structure as *cis*-non-4-ene-6,8-diynoic acid (VII).

Drosophilin F, an acidic ene-diyne, was the least polar polyacetylene fraction and occurred only in traces (40 mg. from 400 l. of culture medium). Unfortunately chromatography of the methyl esters resulted in decomposition and no further work was possible.

The most polar compounds from the culture medium of *D. subatrata* moved very slowly in the phosphate buffer–ether/benzene mixture and so were well separated from the other metabolic products. They occurred in only very small quantities, some 300 mg. being obtained from 400 l. of culture medium. Moreover, they were accompanied by a great deal of oily, pigmented material which hindered purification. Some improvement was effected by alumina chromatography of the methyl esters, and careful re-chromatography of the material eluted with benzene revealed the presence of four compounds which for convenience were designated by their longest-wavelength absorption maxima.

The first eluted compound had maxima at 2950, 2780, and 2650 Å, but as less than 1 mg. of material was isolated no further work was possible. The next fractions contained a mixture of two compounds (longest-wavelength maxima at 3390 and 3040 Å respectively) which were separated by counter-current distribution. The " 3390 " compound was identified as the diester of (VIII), already known to be a fungal metabolite.[10] The " 3040 " compound proved to be the diester of the hitherto unknown diacid (IX). It possesses the diyne-ene-carbonyl chromophore, and infrared examination showed the presence of both conjugated and unconjugated ester groupings and *trans*-ethylenic hydrogen. Chain-length determination on a micro-scale posed a new problem which was solved by conversion into the dimethyl ether of the saturated diol and comparison with authentic specimens by gas chromatography. Catalytic hydrogenation was followed by reduction

(VIII) $HO_2C\cdot CH{=}^{t}CH\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}^{t}CH\cdot CO_2H$

(IX) $HO_2C\cdot CH_2\cdot CH_2\cdot CH_2\cdot C{\equiv}C\cdot C{\equiv}C\cdot CH{=}^{t}CH\cdot CO_2H$

with lithium aluminium hydride and methylation of the resultant diol. Gas chromatography showed the dimethyl ether to have a retention time intermediate between those

[9] Celmer and Solomons, *J. Amer. Chem. Soc.*, 1952, **74**, 1870 and subsequent papers.

[10] (*a*) Bu'Lock, Jones, and Turner, *J.*, 1957, 1607; (*b*) Gardner, Jones, Leeming, and Stephenson, *J.*, 1960, 691.

of the dimethyl ethers of 1,10-decanediol and of 1,12-dodecanediol and the same as that of the derivative of 1,11-undecanediol. The diacid (IX) was then synthesised by Mr. R. E. Bew from hexynoic acid and *trans*-bromopentenynoic acid, and its dimethyl ester had properties identical with those of the derivative of the natural acid.

The fourth compound to be eluted had its longest-wavelength maximum at 2820 Å (ene-diyne chromophore). However, counter-current distribution failed to remove a high proportion (75%) of non-polyacetylenic impurity.

This investigation has furnished the first exception to the generalisation made in Part IX,[11] and known to hold good for nearly fifty examples, that fungal polyacetylenes containing an odd number of carbon atoms possess a free ethynyl grouping. The terminal allenic unit in drosophilin D (III) is closely related to and simply derived from the $HC{\equiv}C{\cdot}CH_2$ system, but the C_{11} diacid (IX) is a clear exception. More instances of non-conformity with the rule have now been encountered in work [12] with other fungi.

Experimental

See Part V (*J.*, 1955, 4270) for general directions regarding the handling of these natural polyacetylenes. Evaporations did not need to be carried out under nitrogen in this case.

Ultraviolet absorption spectra were measured for ethanol solutions on a Cary double-beam recording spectrophotometer. Infrared spectra were recorded for carbon disulphide solutions. M. p.s (corrected) were determined on a Kofler block. Alumina for chromatography was Peter Spence grade " H," deactivated by treatment with 5% of 10% acetic acid unless otherwise stated. Gas chromatography of the perhydro-alcohols utilised a column of 5% polyethylene glycol and 2% stearic acid on firebrick (50—90 mesh), with hydrogen as carrier gas at a flow rate of 60 c.c./min. and a flame ionisation detector. The column measured 102 × 0·45 cm. and was kept at 120°. The perhydro-dimethyl ethers were chromatographed on a column of Apiezon L on firebrick (50—90 mesh) with hydrogen at a flow rate of 70 c.c./min. and the same detector. The column measured 100 × 0·45 cm. and was kept at 200°.

Isolation of Polyacetylenes.—The fungus, obtained through the courtesy of Dr. M. Anchel of the New York Botanical Garden, was grown in surface culture on malt medium. During the growth, aliquot parts were withdrawn periodically from sample flasks, and the polyacetylenes present estimated by ultraviolet absorption measurement (at 2805 Å) of an ethereal extract. When the concentration reached a maximum, generally between 28 and 35 days after inoculation, the medium was withdrawn. Several batches were grown and a typical purification procedure is described.

The medium (80 l.) was continuously extracted with ether for 36 hr. The ethereal concentrate, containing 900 mg. of mixed polyacetylenes, was cautiously evaporated and the residue, a thick brown oil, was immediately dissolved in benzene (80 c.c.) and ether (20 c.c.). Insoluble material was removed and the filtrate placed in the first two tubes of a 120-plate Craig-type counter-current distribution apparatus (volumes of upper and lower phases, 40 c.c.). The system used for fractionation was M/15-phosphate buffer of pH 6·64 and 1 : 4 ether–benzene. Generally about 250 transfers were employed. The various polyacetylenes, detected by their light absorption properties, appeared in the eluted fractions in the following order: drosophilin F (10 mg.) after 130 transfers; drosophilin D (220 mg.) after 145 transfers; drosophilin C (350 mg.) after 180 transfers; drosophilin E (140 mg.) after 200 transfers. More polar compounds were distributed in the terminal 40 tubes of the apparatus.

The separation of the compounds was sharp except for drosophilins C and E, where several mixed fractions were obtained, their compositions being determined as follows. The two compounds have virtually identical ultraviolet absorption spectra, and use was made of their differing behaviour with alkali in order to follow the separation: 0·1 c.c. of N-sodium hydroxide was added to an aliquot part of an aqueous solution of the fraction under investigation. Drosophilin C is instantly converted into a substance with ene-triyne absorption, *i.e.*, maxima at longer wavelengths than C, whereas drosophilin E remains unchanged.

Drosophilin C (cis-*Undec*-3-*ene*-5,7,10-*triynoic Acid*) (I).—Fractions from the above distribution which contained drosophilin C free from drosophilin E were combined and evaporated and

[11] Jones and Stephenson, *J.*, 1959, 2197; Jones, Pedler Lecture, 1959, *Proc. Chem. Soc.*, in the press.
[12] Bew and Jones, unpublished observations.

the residue redistributed in the same buffer–solvent system in a 50-plate Craig-type apparatus. A sharper distribution curve was obtained and the purest fractions were combined, evaporated to dryness, taken up in ether, and decolorised with a little Norite. The filtered solution which contained *ca.* 100 mg. of drosophilin C was evaporated and the residue taken up in hexane (30 c.c.). The solution was decanted from a small amount of insoluble brown oil and then kept at −40° for 2 hr. Drosophilin C formed colourless needles (80 mg.) which became yellow slowly in light at 20°. Two further crystallisations gave cis-*undec*-3-*ene*-5,7,10-*triynoic acid*, m. p. 97·5—99° (Found: C, 76·7; H, 4·8. $C_{11}H_8O_2$ requires C, 76·75; H, 4·7%), $\lambda_{max.}$ 2805 (ε 15,900), 2645 (ε 19,700), 2505 (ε 13,600), 2380 (ε 7800), 2265 (ε 6100), and 2105 Å (ε 46,800), $\nu_{max.}$ 3300 (free ethynyl hydrogen), 1760 and 1712 (unconjugated CO_2H), 738 and 718 cm.$^{-1}$ (*cis*-ethylenic hydrogen).

Drosophilin C Methyl Ester.—A solution of drosophilin C (20 mg.) and sulphuric acid (0·4 c.c.) in methanol (9·6 c.c.) was kept in the dark at 20° for 3 days. The solution was poured into water and extracted with ether, and the ether solution was thoroughly washed with water and dried. The ester crystallised from hexane at −40° as colourless needles, m. p. below 20°. Its low m. p. precluded analysis. It had $\lambda_{max.}$ 2810, 2655, 2510, 2385, 2265, and 2115 Å, $\nu_{max.}$ 3300 (free ethynyl hydrogen), 1745 (unconjugated ester C=O), 755 and 718 cm.$^{-1}$ (*cis*-ethylenic hydrogen).

Hydrogenation of Drosophilin C.—Drosophilin C (10 mg.) was hydrogenated in ethanol (20 c.c.) with Adams platinum catalyst (43 mg.). Evaporation of the solution yielded undecanoic acid, identified as the *p*-toluidide, m. p. and mixed m. p. 76—78°. A further sample of drosophilin C was hydrogenated as above and the product treated with an excess of lithium aluminium hydride in ether to give undecanol. This was shown to be identical with an authentic sample by gas chromatography.

Drosophilin D (cis-*Undeca*-3,9,10-*triene*-5,7-*diynoic Acid*) (III).—Crude drosophilin D (400 mg.), obtained from several purification runs, was redistributed in M/15 phosphate buffer of pH 7·38 and 9 : 1 ether–benzene in a 120-tube apparatus and 260 transfers were employed. A trace of a new polyacetylene (longest ultraviolet max. 3420 Å) was eluted first, followed by drosophilin D which appeared after 210 transfers. The drosophilin D (300 mg.) in ether (200 c.c.) was extracted with sodium hydrogen carbonate solution (1 × 200 c.c.; 3 × 100 c.c.) followed by acidification, re-extraction into ether, and decolorisation of the ether solution with Norite. Spectrographic assay and evaporation and weighing of aliquot parts showed the drosophilin D to be only 50% pure at this stage. Crystallisation proved difficult as most of the impurity was a brown oil which was rather less soluble than drosophilin D in hexane. The mixture was dissolved in hexane (20 c.c.) and then cooled to −40° for a few minutes. The oil was precipitated, and the supernatant hexane was decanted and kept for 2 hr. at −40°. Drosophilin D then separated as colourless plates (40 mg.). Further crystallisations afforded the pure *cis*-undeca-3,9,10-triene-5,7-diynoic acid, m. p. 22—28°. Light absorption properties and isomerisation behaviour were identical with those of drosophilin D obtained from drosophilin C (see below).

Isomerisation of Drosophilin C (I).—(a) *With sodium hydrogen carbonate solution.* A mixture of drosophilin C and E in ether (500 c.c.) containing 550 mg. of polyacetylenes, obtained by the purification procedure described above, was extracted with saturated sodium hydrogen carbonate solution (2 × 500 c.c.) and the aqueous extract was kept for 5 hr. at 20°. No further change in the ultraviolet absorption spectrum took place after this period. The resulting mixture of drosophilin D and E was resolved between M/15-phosphate buffer of pH 6·64 and 1 : 4 ether–benzene in a 50-plate apparatus. Drosophilin D appeared in the eluted fractions after 60 transfers in 15 fractions, sharply separated from drosophilin E (350 mg.) which appeared after 80 transfers. The drosophilin D (200 mg.) was further purified by extraction into saturated sodium hydrogen carbonate solution followed by acidification and re-extraction into ether. Crystallisation from hexane afforded cis-*undeca*-3,9,10-*triene*-5,7-*diynoic acid* as colourless plates, m. p. 21—28° (Found: C, 76·5; H, 4·7. $C_{11}H_8O_2$ requires C, 76·75; H, 4·7%), $\lambda_{max.}$ 3085 (ε 18,200), 2905 (ε 22,000), 2745 (ε 15,100), 2590 (ε 9000), and 2170 Å (ε 34,900), $\nu_{max.}$ 1960 and 1930 (allene), 1760 and 1715 (unconjugated CO_2H), 838 (terminal allene), 738 and 720 cm.$^{-1}$ (*cis*-ethylenic hydrogen). The methyl ester, which was a liquid, showed identical ultraviolet absorption with that of the parent acid and had infrared carbonyl absorption at 1740 cm.$^{-1}$. Hydrogenation (platinum) of drosophilin D followed by reduction with lithium aluminium hydride gave undecanol, identified by gas chromatography.

(b) *With sodium carbonate solution.* Drosophilin C (46 mg.) in ether (35 c.c.) was extracted into 10% sodium carbonate solution (35 c.c.) and left for 2 hr. at 20°. No change in the ultraviolet absorption spectrum occurred after this period. The solution was acidified and the product isolated *via* ether. cis-*Undec*-3-*ene*-5,7,9-*triynoic acid* (IV) crystallised from methylene chloride–hexane at −40° as pale yellow needles, m. p. 115—120° (decomp.) (Found: C, 76·1; H, 4·5%; equiv., 172. $C_{11}H_8O_2$ requires C, 76·75; H, 4·7%; equiv., 172), $\lambda_{max.}$ 3295 (ε 12,000), 3075 (ε 17,600), 2885 (ε 13,300), 2725 (ε 7170), 2575 (ε 3920), 2410 (ε 97,500), 2300 (ε 63,800), 2105 (ε 37,600), and 2040 Å (ε 37,000), $\nu_{max.}$ 1760 and 1710 (unconjugated CO_2H), and 740 cm.$^{-1}$ (*cis*-ethylenic hydrogen). The methyl ester, after chromatography on alumina, formed rosettes of needles, m. p. 27—31°. It showed ultraviolet light absorption identical with that of the parent acid, and infrared carbonyl absorption at 1745 cm.$^{-1}$.

Hydrogenation (platinum) of the acid gave undecanoic acid, identified as the *p*-toluidide, m. p. and mixed m. p. 77·5—79°.

Isomerisation of Drosophilin D (III).—Drosophilin D (19 mg.) was dissolved in 10% sodium carbonate solution (30 c.c.). After 2 hr. at 20° the solution was worked up in the usual way. The product crystallised from methylene chloride–hexane and was shown to be identical in m. p. and ultraviolet and infrared absorption spectra with *cis*-undec-3-ene-5,7,9-triynoic acid (IV).

Drosophilin D (5 mg.) was hydrogenated (platinum), and the product reduced with lithium aluminium hydride to undecanol, identified by gas chromatography.

Drosophilin E (cis-*Non*-4-*ene*-6,8-*diynoic Acid*) (VII).—The purification of this constituent has been described under the isomerisation of drosophilin C with sodium hydrogen carbonate solution (see above). cis-*Non*-4-*ene*-6,8-*diynoic acid* crystallised from hexane as light-sensitive prisms, m. p. 35° (Found: C, 72·2; H, 5·6. $C_9H_8O_2$ requires C, 72·95; H, 5·45%), $\lambda_{max.}$ 2795 (ε 13,000), 2640 (ε 15,100), 2500 (ε 11,600), 2380 (ε 6950), 2270 (ε 4650), and 2100 Å (ε 35,800), $\nu_{max.}$ 3300 (free ethynyl hydrogen), 1717 (unconjugated CO_2H), and 740 cm.$^{-1}$ (*cis*-ethylenic hydrogen). Hydrogenation (platinum) and reduction by lithium aluminium hydride gave nonanol, identified by gas chromatography.

The Polar Compounds.—The accumulated total of polar polyacetylenes from 400 l. of culture fluid amounted to approx. 300 mg. This material was esterified with 5% sulphuric acid in methanol (100 c.c.) for 3 days at 20°. The mixture was then poured into water (500 c.c.), isolated *via* ether, and chromatographed on alumina (50 g.) in benzene. Elution with benzene gave a series of fractions which were obviously mixtures; these were recombined and resolved further (see below). Ether eluted material showing ene-diyne absorption but repeated chromatography failed to effect purification.

The benzene eluates were carefully rechromatographed on alumina (50 g.). Light petroleum–benzene (9 : 1) eluted traces of a compound with ultraviolet maxima at 2950, 2780, and 2650 Å, but there was insufficient material for further work. Light petroleum–benzene (1 : 1) eluted first a mixture of the " 3390 " and " 3040 " compounds and then " 2820 " compound. The first mixture was distributed between light petroleum and 60% aqueous ethanol in a 50-plate Craig-type counter-current apparatus. The " 3390 " compound appeared in the eluted fractions after 62 transfers and the " 3040 " compound after 75 transfers.

The " 3390 " compound crystallised from hexane as off-white needles (10 mg.), m. p. 103—106°, identical with dimethyl *trans*, *trans*-deca-2,8-diene-4,6-diyne-1,10-dioate [the diacid corresponding to (VIII)].[10]

The " 3040 " Compound (*Dimethyl* trans-*Undec*-2-*ene*-4,6-*diyne*-1,11-*dioate, the Diester of* IX).—(*a*) The distribution fractions containing the " 3040 " compound were chromatographed twice on alumina and then crystallisation from hexane afforded the diester as colourless plates (10 mg.), m. p. below 20°, $\lambda_{max.}$ 3040 (ε 19,900), 2860 (ε 20,500), 2700 (ε 12,300), 2550 infl. (ε 6250), 2230 (ε 32,300), and 2160 Å infl. (ε 25,300), $\nu_{max.}$ 1738 and 1725 (unconjugated and conjugated ester C=O) and 955 cm.$^{-1}$ (*trans*-ethylenic hydrogen). The diester (6 mg.) was hydrogenated (platinum) in ethanol, then reduced with lithium aluminium hydride, and the resulting diol was methylated. Potassium (100 mg.) was dissolved in t-butyl alcohol (5 c.c.), and the diol (4 mg.) was added in t-butyl alcohol (2 c.c.). Methyl iodide (1 c.c.) was poured in and the mixture refluxed for 30 min., cooled, and diluted with water. The product was extracted with light petroleum (b. p. 30—40°) which was then thoroughly washed with water and dried. The solution was reduced to small bulk and chromatographed on alumina (Grade H; 15 g.) in 4% ether–light petroleum (b. p. 30—40°). 120 c.c. of eluate were collected and this was then concentrated to dryness. The residue was submitted to gas chromatography and had a retention

time of 6·75 min. Retention times for the dimethyl ethers of 1,10-decanediol, 1,11-undecanediol and 1,12-dodecanediol were 4·5, 6·75, and 10 min. respectively.

(*b*) (with R. E. Bew.) A solution of *trans*-5-bromopent-2-en-4-yn-1-oic acid [10b] (102 mg.) in methanol (0·5 c.c.) was added under nitrogen during 10 min. to a stirred solution of hex-5-yn-1-oic acid [13] (64 mg.) and cuprous chloride (3·4 mg.) in 33% aqueous ethylamine (1·0 c.c.) at 30°. Hydroxylamine hydrochloride was introduced in small portions as the reaction proceeded, to maintain the copper in the reduced form. After a further 10 minutes' stirring, dilute hydrochloric acid (10 c.c.) was added and the acid (60 mg.) was isolated *via* ether. Crystallisation from ether–hexane gave pale yellow crystals of trans-*undec*-2-*ene*-4,6-*diyne*-1,11-*dioic acid* (IX), softens at 130°, m. p. 157—159° (decomp.) (Found: C, 64·6; H, 4·6. $C_{11}H_{10}O_4$ requires C, 64·1; H, 4·85%), $\lambda_{max.}$ 3020 (ε 18,500), 2840 (ε 19,300), 2680 (ε13,200), 2540 infl. (ε 7900), 2215 (ε 35,300), and 2150 Å infl. (ε 29,800), $\nu_{max.}$ (in CCl_4) 3480 (CO_2H), 2230 (C≡C), 1710 (CO_2H), 1620, 1137, and 960 cm.$^{-1}$ (*trans*-ethylenic hydrogen). Treatment with methanolic 5% sulphuric acid (30 c.c.) overnight at 20° and isolation *via* ether gave the *dimethyl ester* which after chromatography on alumina and crystallisation from hexane formed crystals, m. p. 15—16° (Found: C, 66·0; H, 6·1. $C_{13}H_{14}O_4$ requires C, 66·65; H, 6·0%), $\lambda_{max.}$ 3040 (ε 19,500), 2860 (ε 19,600), 2700 (ε 11,500), 2550 infl. (ε 5450), 2225 (ε 35,700), and 2160 Å infl. (ε 27,650), $\nu_{max.}$ identical with those of diester from natural acid.

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[13] Eglinton and Whiting, *J.*, 1953, 3052.